

CHANGES IN pH OF ALBUMEN AND YOLK IN THE
COURSE OF EMBRYONIC DEVELOPMENT
UNDER NATURAL AND ARTIFICIAL
INCUBATION.

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Considering the influence of acidity on colloidal properties of organic substances, such as animal fluids, tissues, etc., it is important to recognize the significance of pH value in various parts of the developing bird's egg. The most essential parts of the egg are albumen and yolk; they furnish almost all the food material to the growing embryo during the entire period of incubation. They must have a proper pH value. There is evidence to warrant the belief that any variation in pH of albumen and yolk from a normal value, particularly when affected by an environment, such as carbon dioxide gas, will develop a pathological condition within an egg and in extreme cases, will cause the death of the embryo. Therefore, it is important to establish the normal curves of pH value for both egg constituents in order to assist in further comparative studies of the growth and metabolism of the embryo under various environmental conditions of artificial incubation.

The literature relating to the subject shows that Aggazzotti, ('13), presumably, was the first who observed pH value of albumen and yolk in incubated eggs. Then a few observations were made by Gueylard ('25), Healy and Peter ('25), and, on egg albumen alone, by Wladimiroff ('26). Unfortunately, the data of the above investigators, being either insufficient or inconsistent, do not give a complete picture of all changes in pH value of the egg constituents during the incubation period.

The present paper concerns itself with a study of pH value of albumen and yolk in the developing egg throughout the incubation period under both natural and artificial (experimental) incubation.

METHODS AND MATERIALS.

The eggs used were from White Leghorn hens. They were selected for uniformity of size, shape, and shell texture, and incubated soon after laying (in spring, 1929). In one experiment the eggs were incubated by the natural method, under sitting hens; in another experiment, by an artificial method, in a special electric laboratory incubator (Romanoff, '29). All physical factors of artificial incubation were predetermined and kept constant. The temperature was $38.0 \pm 0.2^\circ \text{C}$.; the relative humidity 62.5 ± 1.0 per cent; the inside ventilation was 0.5 cu. ft., and fresh air from outside was added to give an adequate supply of oxygen. The carbon dioxide content was increasing from 0.15 to 0.65 per cent. The eggs were turned three times a day.

In the experiments, at intervals of twenty-four hours, usually four eggs with normally-developed embryos were removed for analysis. The pH values of egg albumen and yolk were determined electrometrically, using hydrogen electrode. The observation of yolk was carried on up to the hatching time, and that of albumen up to the sixteenth day of incubation, as long as it was available. After that time the egg albumen normally enters the yolk sac and loses its physical appearance.

RESULTS AND DISCUSSION.

In a study of fresh eggs (just laid, warm, and without an air cell) it was found that the pH value of albumen and yolk was on an average 7.827 ± 0.046 and 5.973 ± 0.015 . A slight difference was noticed in pH value of the outer and middle layers of egg albumen, examined separately. This is illustrated by the data of a few analyses (Table I.).

TABLE I.
HYDROGEN-ION CONCENTRATION IN FRESH EGGS.

Egg Number.	Layers of Egg Albumen.		Egg Yolk.
	Outer.	Middle.	
	pH	pH	pH
1.....	7.929	7.890	5.960
2.....	7.995	7.951	5.977
3.....	8.001	7.946	5.968
Average.....	7.975	7.929	5.968

The middle layer, being close to the yolk (Fig. 1), was invariably less alkaline than the outer one. In incubation as soon as an egg is heated the different layers of albumen disappear, and the separ-

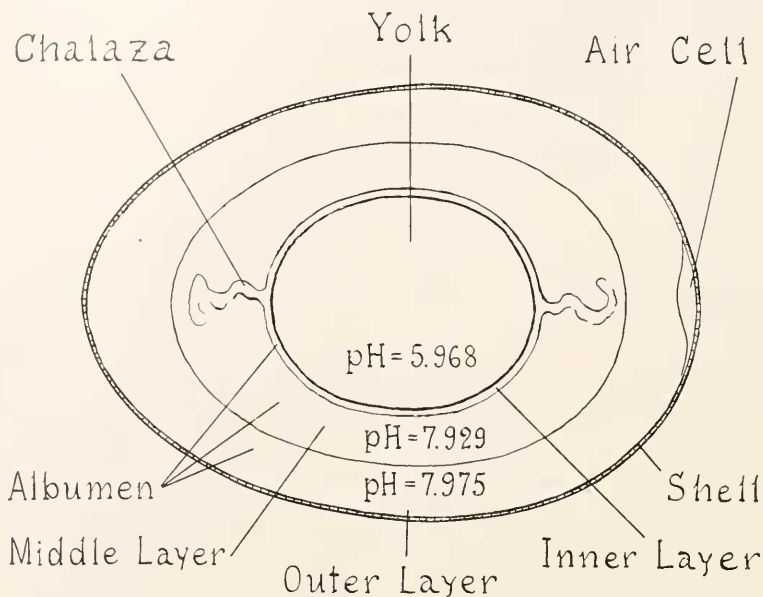


FIG. 1. Hydrogen-ion concentration of a fresh egg.

ate analyses can not be performed. No attempt was made to determine pH value of the inner layer, because of its extremely small size.

The data of our experiments on pH value of egg albumen under natural and artificial incubations, together with the data of previous workers, are shown in Table II.

It is evident, particularly from our data (Fig. 2), that egg albumen rapidly becomes alkaline, reaching the highest point at about two days of incubation. Then it turns towards neutrality, reaching the original pH value at about six days of incubation. A slight difference in pH value at two days, under natural and artificial methods, may be explained primarily by the influence of environmental conditions of incubation. Presumably, the carbon dioxide concentration was lower in the laboratory incubator than under the sitting hen. This difference can not exceed 0.1 per cent. of carbon dioxide concentration.¹

¹ A paper on "Effect of Carbon Dioxide on pH of Albumen in the Developing Egg," has been prepared for publication. (Will appear in *Jour. Exp. Zoo.*.)

The data of our experiments on pH value of egg yolk under both methods of incubation, with the data of other workers, are shown in Table III.

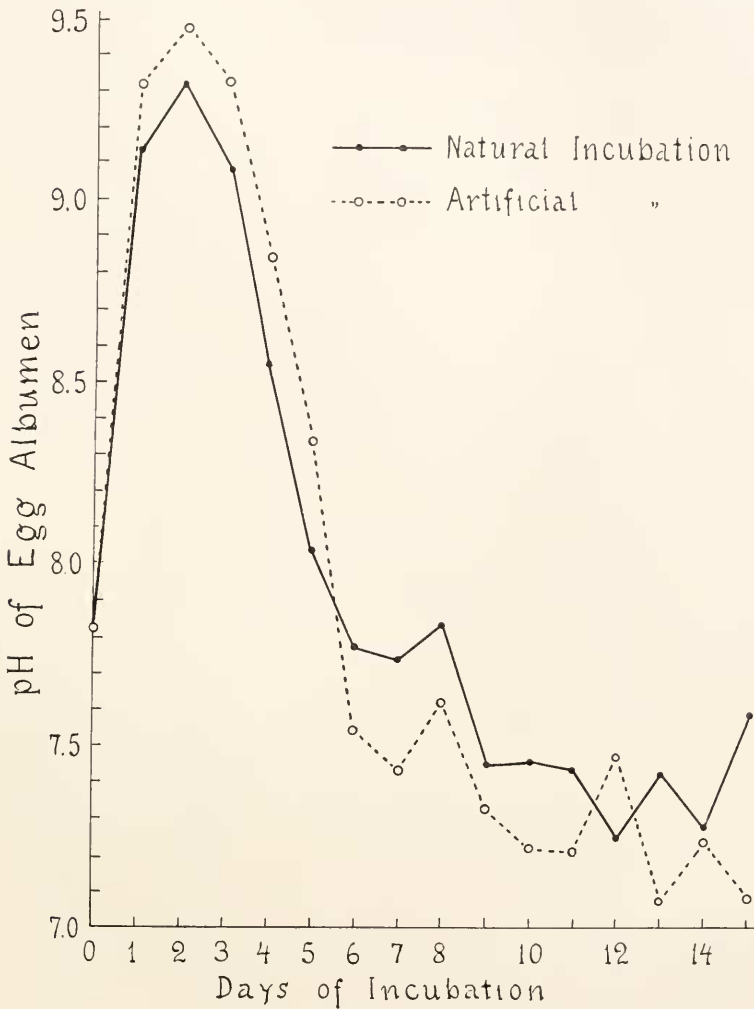


FIG. 2. Changes in pH of egg albumen under natural and artificial (experimental) incubation.

The pH value found by Aggazzotti ('13) seems to be consistently lower than ours. His data, being incomplete, do not demonstrate the pronounced drop at 16 days of incubation. It is seen

TABLE II.
CHANGES IN pH OF EGG ALBUMEN DURING INCUBATION.

Incub. Age.	Aggazzotti (1913).	Gueylard & Portier (1925).	Healy & Peter (1925).	Wladimiroff (1926).	Authors.	
					Art. Incub.	Nat. Incub.
(days)	pH	pH	pH	pH	pH	pH
0	8.66	7.97	8.24		7.827	7.827
1	8.34				9.317	9.157
2	8.38	8.60		9.36	9.472	9.311
3	7.91		9.4	9.46	9.318	9.080
4	7.40			9.19	8.835	8.546
5	7.82			8.72	8.343	8.038
6	7.41		8.2	8.66	7.536	7.785
7	7.71			8.57	7.414	7.748
8	7.24			7.88	7.614	7.840
9	7.34			8.29	7.328	7.450
10		7.25		7.82	7.255	7.466
11	6.61			7.88	7.231	7.435
12				7.55	7.473	7.257
13				7.47	7.079	7.419
14	6.14				7.247	7.283
15				7.57	7.086	7.593
16				7.61		

TABLE III.
CHANGES IN pH OF EGG YOLK DURING INCUBATION.

Incub. Age.	Aggazzotti (1913).	Gueylard & Portier (1925).	Healy & Peter (1925).	Authors.	
				Art. Incub.	Nat. Incub.
(days)	pH	pH	pH	pH	pH
0	4.06	5.48	6.36	5.973	5.973
1	4.83			6.087	6.034
2	4.04	5.73		6.151	6.082
3	4.37		6.8	6.215	6.234
4	5.55			6.286	6.117
5	5.73			6.445	6.541
6	6.12	7.54	6.8	6.631	6.589
7	5.99			6.667	6.872
8	5.96			6.756	6.946
9	5.85			6.750	7.007
10		6.83		6.855	7.327
11	6.68			6.926	7.430
12				7.050	7.450
13	6.82	7.42		7.043	7.772
14	6.98	7.08		7.042	7.920
15				6.899	8.099
16				6.105	7.401
17	6.83	4.12		7.693	7.820
18	6.26	5.64		7.701	7.637
19	6.64			7.714	7.608

from the curves of pH value under natural and artificial incubation (Fig. 3) that egg yolk, from its original acid state, gradually goes to a neutral, and then to a slightly alkaline state. The

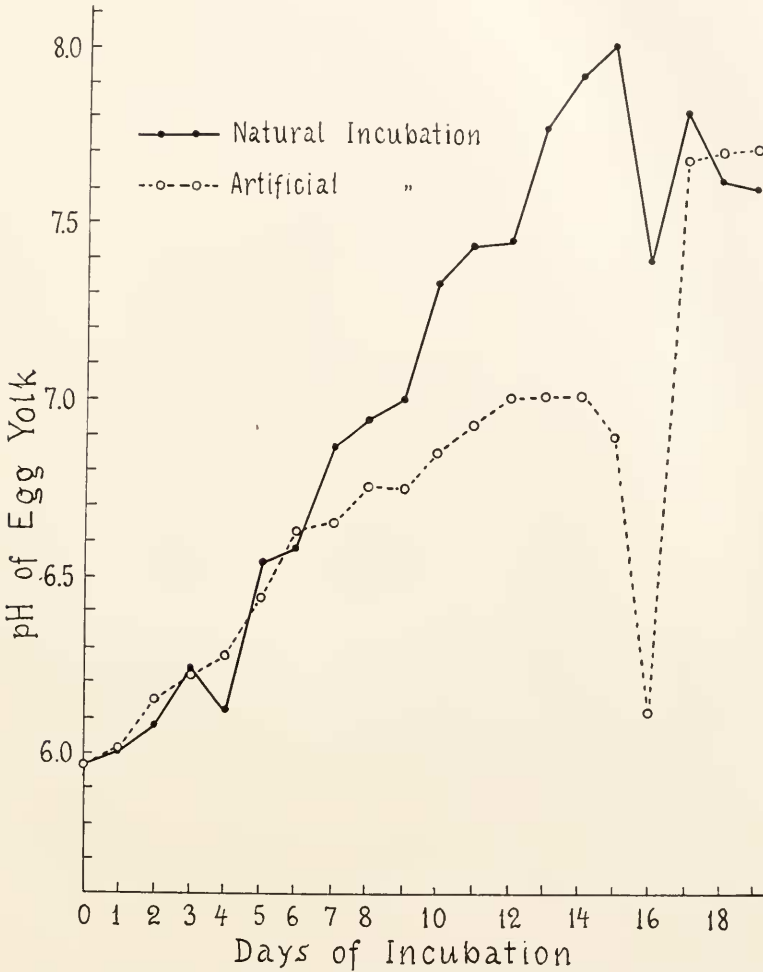


FIG. 3. Changes in pH of egg yolk under natural and artificial (experimental) incubation.

above-mentioned sudden drop in pH value under both natural and artificial incubation is possibly related to the natural depression of the growth in the life span of the embryo (Romanoff, '29a). But the difference in pH value under natural and artificial meth-

ods during the period from 7 to 16 days of incubation may be explained, as suggested above by the difference in carbon dioxide concentration.

SUMMARY.

1. In the course of embryonic development the pH values of albumen and yolk go through definite changes, presumably affected by natural metabolic processes occurring within an egg.

2. It was found that the changes in pH either of albumen or of yolk were similar under natural and artificial (laboratory) methods of incubation.

3. The pH of egg albumen rapidly changed towards alkalinity and back during the first week of incubation, reaching the highest point of alkalinity at about 48 hours. For the rest of the incubation period it gradually moved towards neutrality.

4. The pH of egg yolk gradually changed throughout the incubation period from acid to alkaline, with a sudden temporary drop at the sixteenth day.

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